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Original Research Article

Clinico-mycological profile of Dermatophytosis In a Tertiary Care Hospital in West Bengal – An Indian scenario

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Keywords

Dermatophytosis Tinea unguium, Trichophyton, Microsporum, Epidermophyton.

A B S T R A C T

358 clinically suspected cases of dermatophytosis were subjected to mycological examination with direct microscopy using 10%-40% KOH depending on the type of sample processed and culture on Sabouraud's Dextrose Agar with chloramphenicol (SDCA), Sabouraud's Dextrose Agar with chloramphenicol and cyclohexamide (SDCCA) and also in Dermatophyte Test Medium (DTM).Causative agents were identified macroscopically and microscopically from the growth obtained on SDCA and SDCCA. Direct microscopy revealed fungal element in 327(91.34%) cases whereas 313(87.43%) were positive on culture. Commonest age group affected were between 31-40 years (25.69%). Males were predominantly affected (52.51%) and male to female ratio being 1.1:1. 57% of patients came from urban background compared to 43% from rural areas. Housewives formed the largest group being 29% followed by labourers (21%) and farmers (16%). 48%patients gave history of possible source of infection..Other uncommon species of *Trichophyton* isolated were *Trichophyton concentricum*,*Trichophyton simii* and *Trichophyton equinum.* Among different *Microsporum* species identified, *Microsporum gypseum* (5.29%) was the commonest isolate followed by *Microsporum audouinii* (4.23%) and *Microsporum canis*(1.58%) infrequently. Few rare species like *Microsporum praecox*, *Microsporum nanum* and *Microsporum ferrugenium* were also identified in this study. Among Genus *Epidermophyton*, *Epidermophyton floccosum* (3.17%) and *Epidermophyton Stockdale* (1.05%) were isolated. DTM was found useful as a general screening medium for dermatophytes.Both SDCA & SDCCA were found to be equally effective in isolating dermatophytes from clinical samples in our study.

Introduction

Dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissues (hair, skin & nail) of anamorphic class Hyphomycetes of the Deuteromycota (Fungi Imperfecti)[3]. Dermatophytes typically do not affect the

mucous membranes rather affects keratinized tissues and spread by direct contact with infected human beings (anthropophilic organisms), animals (zoophilic organism), soil (geophilic organisms) and also by indirect way from fomites[4]. Traditionally infections caused by dermatophytes (ringworm) have been named according to the anatomical locations involved, by appending the Latin term designating the body site after the word 'Tinea' [5].

Dermatophytosis is a major public health problem in the world today. Dermatophytosis is common in tropical countries like India and may reach epidemic proportion in areas with high rate of humidity, over population. and poor hygienic conditions [6]. The disease is more frequent among men than woman. Several factors have been implicated to increase in disease such as trauma, increased sweating and diabetes [7].

Despite increasing reports of dermatophytoses in different tropical and subtropical countries, there is scanty data on this issue from India especially from West Bengal which is situated in the Eastern part of India.

The present study was undertaken with the objectives include to determine the incidence, contributing factors associated with dermatophytoses and occupational consequences related to disease. Also to isolate and characterise the causative dermatophytes and the species prevalent in this part of country.

Materials and Methods

This is a cross-sectional and observational study over a period of two and a half (2·5) years from September 2011 to February 2014 conducted at R.G.Kar Medical College & Hospital, Kolkata in West Bengal.

The study population comprised of 358 clinically suspected cases of dermatophytoses attending Dermatology outpatients department at R.G.Kar Medical College & Hospital, Kolkata during a period of two and a half (2·5) years i.e from September 2011 to February 2014. Our hospital which is a tertiary care hospital caters patients from densely populated metro city of Kolkata along with a wide suburban area surrounding it. Majority of the people are from rural areas with low socioeconomic background and poor literacy rate.

Detailed history of onset of disease, duration of symptoms, trauma, occupation, drugs, associated co morbid conditions, family and personal history was taken. Enquiries were also made as to exposure to animals, cases or any other suspected sources.

Collection and processing of the sample

Samples were collected from affected lesions. Whenever the patients presented with lesions at clinically different sites samples were collected from all those sites and each of these were processed and examined individually.

Collection of samples from skin:

The affected area was swabbed with 70% alcohol and the active edge of lesion scraped with a flame sterilized blunt scalpel. The scrapings were collected from active margin of lesion without injuring the skin surfaces.

From the nails

The affected nails were swabbed with 70%

alcohol after which the nails were scraped deeply enough to obtain recently affected nail tissue. Nail clippings were also collected in addition to nail scrapings from the lesions whenever it is feasible.

From the scalp

The same procedure as mentioned for skin scrapings was followed, in addition few affected hairs were also epilated and collected with a pair of flame sterilized forceps. Care was taken to collect the basal portion of hair (hair stub) as the fungus was usually found in this area. If it is not possible due to hair fragility as in 'black dot' in 'Tinea capitis', a sterile scalpel was used to scrape scales surrounding the hair root and excavate the remaining portions of the hair root.

The nail clippings and hair samples were cut into small fragments of 1mm in size. Out of the material collected, part of it was used for direct KOH examination and remaining part was used to inoculate onto Sabouraud dextrose agar with chloramphenicol (SDCA), Sabouraud dextrose agar with chloramphenicol & cyclohexamide (SDCCA) and Dermatophyte test medium (DTM) with supplement to isolate the causative dermatophytes.These three culture media used in our mycology laboratory were obtained as dehydrated media (manufacturer – HiMedia Laboratories, Mumbai) and prepared in-house following stringent quality control measures. DTM is a selective medium recommended for the isolation and cultivation of pathogenic dermatophytic fungi. It is a modification of a commercial formulation made by Taplin et al in 1969[6,8].

KOH examination

Skin and hair specimens were subjected to 10% KOH solution. The preparation was kept at room temperature for 30 mins. Nail clippings and scrapings were kept overnight dipped in 40% KOH solution. Subsequently examination was done under low power objective (10x) of the microscope for branching and septate hyphae and confirmation was made by high power objective (40x) of it.

Culture

Skin, hair and nail samples were inoculated after reducing the size of the samples to approximately to 1 mm as it was mentioned earlier. Inoculations were done at four (4) sites at well spaced interval onto Sabouraud's dextrose agar slants with chloramphenicol (0.05mg/ml) and cyclohexamide (0.5mg/ml)[6,9]. Chloramphenicol was added to inhibit the growth of bacteria and cyclohexamide was used to inhibit the growth of saprophytic fungi. Inoculations of specimens were also done on DTM slopes for isolating dermatophytes where mixed pathogens were suspected. The tubes were incubated in BOD incubator at 28^0c and also at room temperature to achieve good growth of some dermatophytes which prefer a little higher temperature. The tubes were examined at regular intervals for evidence of fungal growth and the progress of growth was also noted. Culture tubes not showing any growth were discarded after six weeks of incubation. Any visible growth on SDCA or SDCCA was examined for colony morphology, texture, pigmentation on surface (obverse), pigmentation on the reverse.

Microscopic examination of colony was done by doing a lactophenol cotton blue mount to examine the hyphal structure, different vegetative structures formed by hyphal modifications, various reproductive

structures like microconidia, macroconidia and chlamydoconidia. Urea hydrolysis was used to distinguish some species of Trichophyton and Microsporum.

Results and Discussion

Among 358 clinically diagnosed cases of dermatophytosis, tinea unguium was the most common clinical types (267/358,74·58%). But dermatophytes were isolated only in 109 cases among 267 clinically diagnosed onychomycosis cases. The other onychomycotic cases had etiology of non-dermatophytic molds. The study group comprised of (358 clinically diagnosed cases of dermatophytosis) 188(52·51%) males and 170(47·48%) females [Fig-2]. Male outnumbered female with a ratio of 1·1:1. The commonest age group affected was 31-40 yrs (25·69%) followed by 41-50 years (19·83%) [Table-1]. Dermatophytosis were commonest among housewives (29%) followed by labourers (21%) and farmers (16%).

48% of cases gave history of contact with possible sources of infection like history of contact with cases 13%, history of contact with animals 22%, history of both contact with animals & cases 5% and history of similar episodes in the past 8%. History of trauma was also important in 23% cases especially in tinea unguium. This confirms that dermatophyte infections are transmitted from person to person by sharing common household things and fomites [6].Co-morbidity was noted in 18% of clinically diagnosed cases. The commonest co-morbid condition being diabetes mellitus (Type-2) followed by atopy (asthma, eczema) and alcoholic liver disease.

Duration of symptoms ranged from 15 days to 5 years, mean duration being 2·5 months. Tinea unguium was the most common lesion accounting for 74·58% of cases followed by Tinea capitis 8·93%, Tinea corporis 8·65%, Tinea pedis 3·35% and Tinea cruris 3·07%. Other clinical types like Tinea manum and Tinea faciei were found as rare types and only in 0·83% & 0·55% of cases [Table-1].

Direct microscopy (KOH mount) revealed fungal hyphae in 327(91·34%) samples whereas culture positivity was found in 313(87·43%) samples. Out of 313 culture positive samples 11 were negative on microscopy (KOH mount). Thus in 20/358 (5·58%) samples showed no evidence of fungi either on direct microscopy or by culture [Table-2]. It is noteworthy that lesions in the mixed sites (mixed clinical types) were not observed in any case.

It is noteworthy that, three rare species of Trychophyton were isolated from three different clinical types of dermatophytosis which were *Trichophyton concentricum, Trichophyton simii* and *Trichophyton equinum* from cases of tinea unguium, tinea corporis and tinea pedis respectively.

The epidemiology of superficial fungal infections has changed significantly in the last century and reflects changes in socioeconomic conditions, lifestyles and migration. Few studies have investigated the etiology of superficial fungal infections in the developing world, and consequently, there is less knowledge of changes in their epidemiology [10, 11]. It is difficult to ascertain reliably the overall incidence and prevalence of the various skin diseases caused by superficial mycoses in different parts of the world because studies of one region of a country may not be a true representation of the overall disease pattern of that country; furthermore, incidence and prevalence figures may only be representative of the population sampled, which may have associated risk factors for infection [12].

Dermatophytes are widely prevalent in our part of world. The higher incidence of dermatophytosis could be attributed to environmental conditions. In the present study, dermatophytosis was found to be commonest in the age group 31-40 yrs which is in contrast to others [6,13,14]. Higher incidence was noted amongst males (52·51%) than in females (47·48%), ratio being 1·1:1 which well with most of the studies. Higher incidence in males may be because they are exposed to outdoor with greater physical activity and more prone to trauma [15].

57% patients came from urban background compared to 43%came from rural areas which can be explained as urban patients seek medical advice sooner due to awareness and accessibility of medical care [13, 16].

Housewives formed a major chunk of cases (29%) followed by labourers (21%) and farmers (16%) in accordance with most of studies [13, 16, 17]. Predominant clinical types noticed among housewives were 'Tinea unguium' which may be correlated with their wet occupations. Regarding duration of disease, 65% patients had clinical symptoms persisting for more than six (6) months suggesting the chronicity of nail, hair and skin infections [13, 18].

In the present study most common association was found with atopy followed by diabetes mellitus which is in conformity with other reports[13,19] but no concomitant tinea infection was noted which is in contrast to other studies[20].

327/358 (91·34%) were positive by direct microscopy and 313/358 (87·43%) were culture positive which is close to the findings of Balakumar Srinivasan et al[4] but does not corroborate with majority of the studies[6,13,21]. In the present study high positivity both in direct microscopy (KOH mount) and culture reflects technical skill along with use of good quality culture media prepared following stringent quality control measures. Moreover the selection of cases with accuracy in clinical diagnosis may be an added factor.

Tinea unguium(74·58%) was observed as the most common clinical condition followed by Tinea capitis(8·93%) and Tinea corporis(8·63%) which differs from most of the studies conducted in different parts of India where the commonest clinical types observed were Tinea cruris[6] and Tinea corporis[4,13]. Among various clinical conditions, Tinea capitis was common in children below the age of 12 years. This finding in our study corroborates well with others [4, 6].

The most common fungal isolate was Trichophyton verrucosum (23.8%) followed by T.rubrum (22.2%), T.mentagrophyte (21.16%), T.schoenleinii (6.34%), T.saudanense (4.76%) and T.violaceum (2.11%). Overall Trichophyton was the most common genus (82.01%) which was in accordance with other studies [22, 23].It is noteworthy that three uncommon species of genus Trichophyton namely T. concentricum, T.equinum and T.simii, an unusual aetiology of dermatophytosis were also identified during this study [Table-3].

In the present study, among the different species of genus Microsporum(13.7%) isolated, Microsporum gypseum(5.29%) was found as the most common isolate followed by M. audounii(4.23%) and M. canis(1.58%).Some rare species like M.nanum, M.praecox and M. ferrugenium were identified in this study[Table-4].

Genus Epidermophyton constitutes 4.23% of the total dermatophytes isolated in this study

among which E.floccosum (3.14%) were predominant species. But it is interesting to note that E.stockdaleae had also been isolated from Tinea unguium and Tinea corporis, one from each clinical type [Table-5].

Most of the isolates obtained were anthropophilic dermatophytes of Genus Trichophyton, Microsporum and Epidermophyton which reflects the source of infection are from cases. However Trichophyton verrucosum was the predominant isolate which is a zoophilic fungus that signifies contact of people with donmestic and pet animals intensely, especially in rural Bengal.

Present study also showed the higher isolation of M.gypseum (geophilic dermatophytes) which could be accounted due to patient's interaction with soil and domestic animals [24]. Ranganathan et al reported isolation of M.gypseum from the dermatophytosis of domestic and pet animals in and around Chennai [25].

The present study gave an insight about the etiological agents of dermatophytosis in this part of West Bengal where the climatic condition, occupation, socioeconomic conditions, low literacy rate and lack of knowledge about the disease plays crucial role in causation and chronicity of the disease. In case of commonest lesion, species isolated and other variables it differs from other part of India. DTM is more useful as a general screening medium and the isolation of dermatophyte is also rapid compared to SDCA & SDCCA which are found equally effective identification media in our Mycology laboratory.

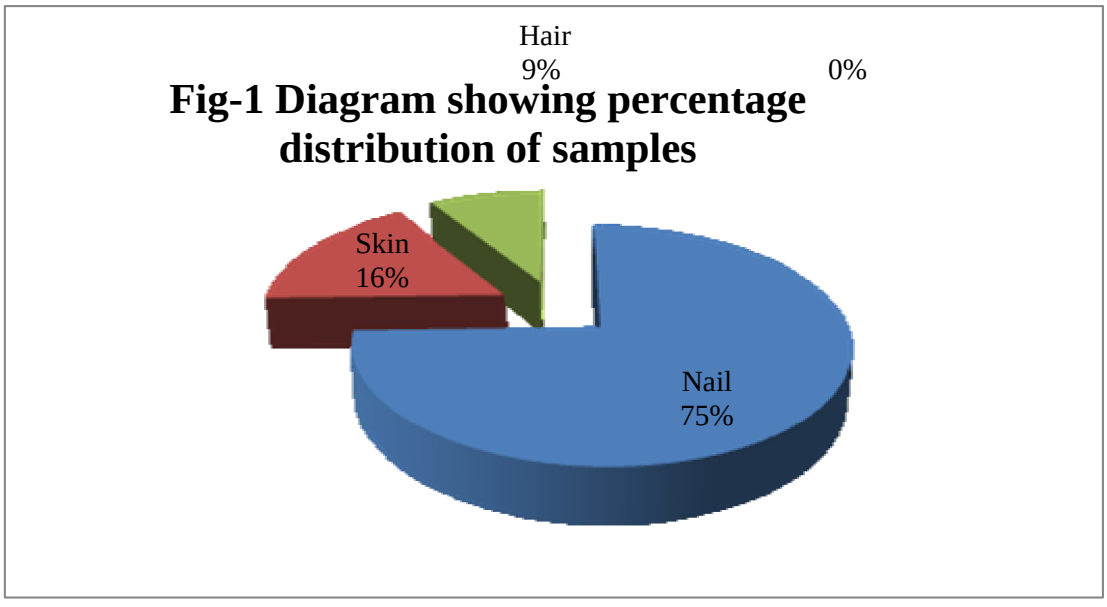

Fig-1 Diagram showing percentage distribution of samples

Table.1 Dermatophytosis with reference to clinical manifestation versus age group n, (%)

Clinical manifestation	Total no. of samples n= (%)	Age 0-10 yr. n= (%)	11-20 yrs	21-30 yrs	31-40 yrs	41-50 yrs	51-60 Yrs	>60 yrs
T. unguium	267(74·58)	04(1.49)	18(6.74)	38	67(25·0)	59(22·0)	47(17·6)	34(12·73)
T.capitis	32 (8·93)	18(56·5)	7	03	00	02	00	02
T. corporis	31 (8·65)	00	02	0	12	10	03	04
T. pedis	12 (3·35)	00	00	03	05	00	02	02
T. cruris	11 (3·07)	00	00	01	07	00	03	00
T. manum	03 (0·83)	00	00	02	01	00	00	00
T. faciei	02 (0·55)	00	01	00	00	00	01	00
Total	358	22(6·14)	28	47	92(25·6)	71(19·8)	56	42

Tinea unguium(T.unguium), Tinea capitis(T.capitis), Tinea corporis(T. corporis),Tinea pedis(T.pedis),Tinea cruris(T.cruris), Tinea manum(T.manum), Tinea faciei(T.faciei).

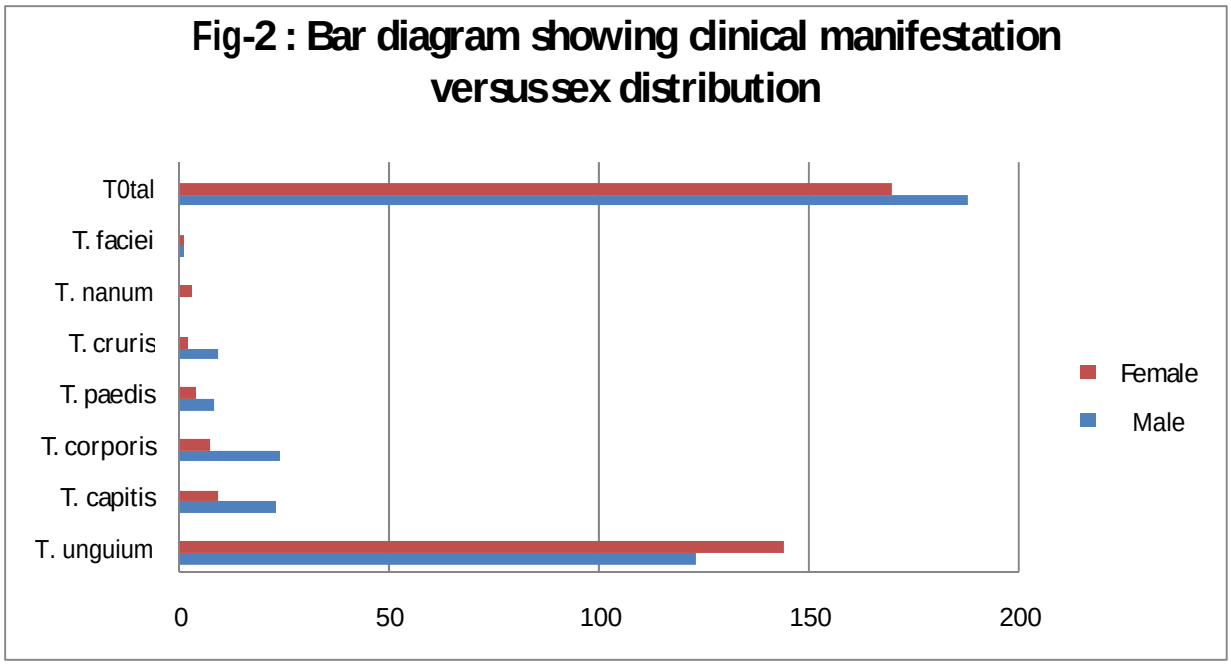

Table.2 Microscopy and culture positivity of 358 clinical samples

	Samples n= (%)
Total KOH positive	327 (91·34)
KOH positive & culture negative	25 (6·98)
KOH negative & culture positive	11 (3·07)
Total culture positive	313 (87·43)
Both positive	302 (84·35)
Both negative	20 (5·58)

Table.3 Dermatophytes (Trichophyton species) isolated from different Clinical types: n (%)

Clinical types ↓	**Species→** ***T.verrucosu--m***	***T.rubrm***	***T.mentagrophyte***	***T.schoenleinii***	***T.saudonense***	***T.violaciu--m***	**Total**
T.unguium	40	21	30	4	7	1	103
T.capitis	0	4	1	2	2	1	10
T.corporis	3	9	4	3	0	0	19
T.pedis	1	3	2	2	0	1	9
T.cruris	1	4	3	0	0	0	8
T.manum	0	1	0	0	0	0	1
T.faciei	0	0	0	1	0	1	2
Total	45(23·80)	42(22·2)	40(21·16)	12(6·34)	9(4·765)	4(2.11)	152

T.concentricum-1, ***T.equinum***-1, ***T. Simii***-1 **Total** =155(82·01%)

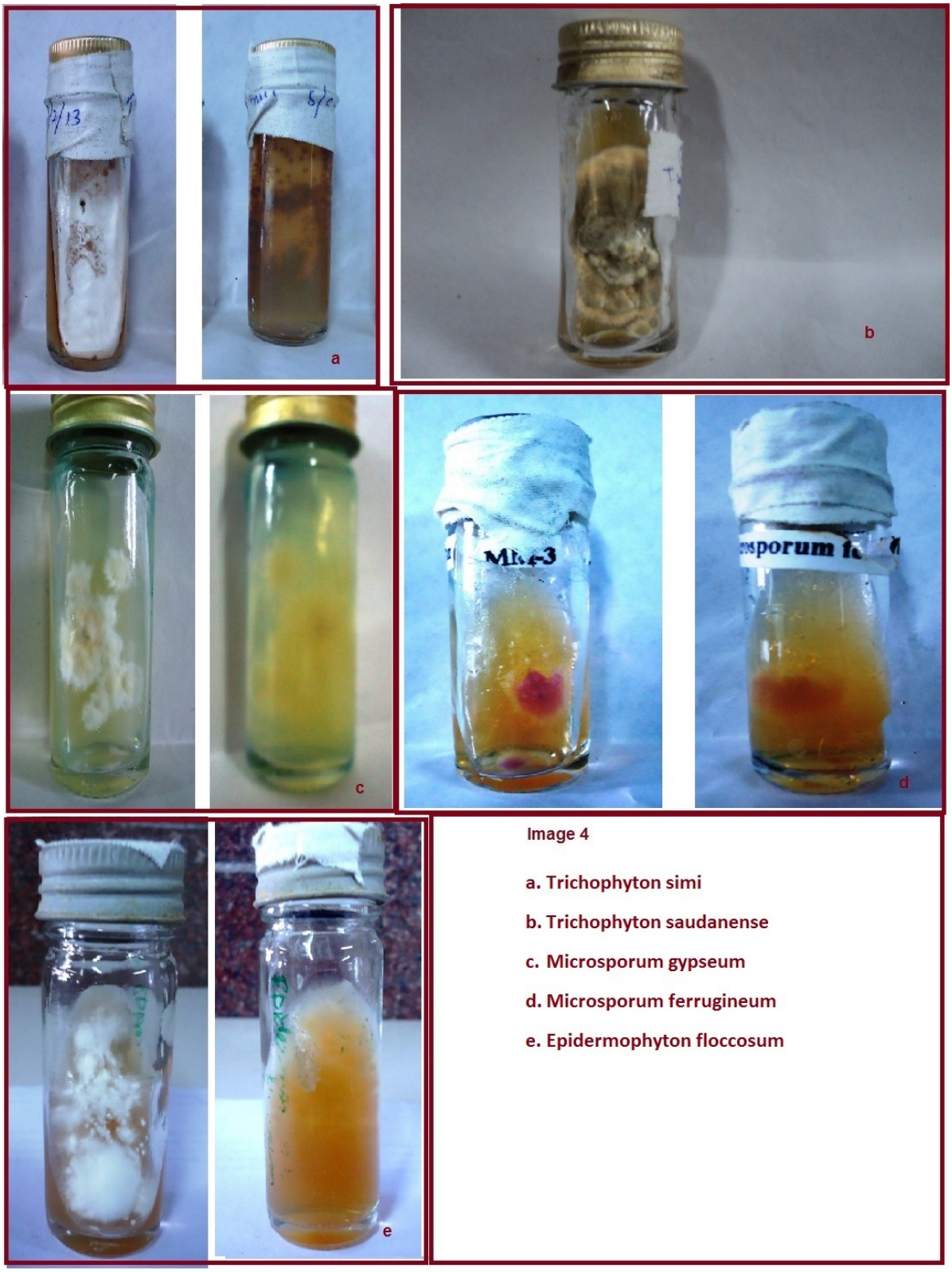

Image 4

a. Trichophyton simi

b. Trichophyton saudanense

c. Microsporum gypseum

d. Microsporum ferrugineum

e. Epidermophyton floccosum

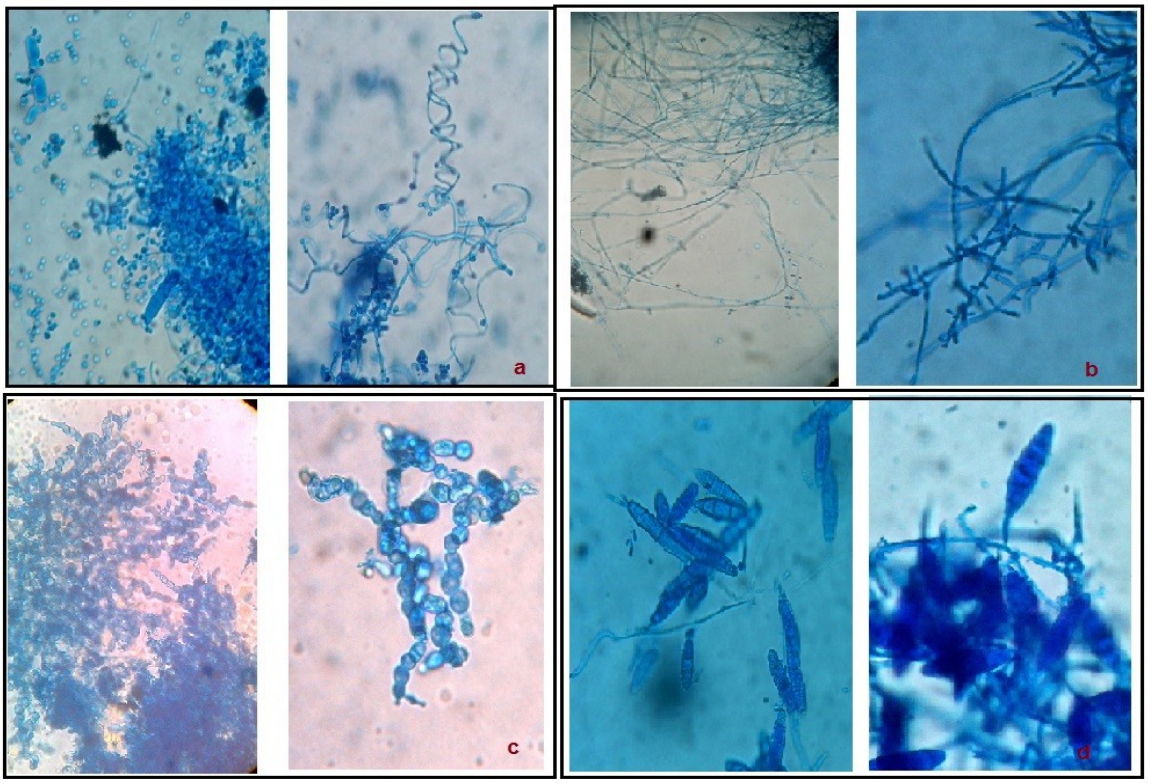

Image :

a) LPCB mount showing microscopic morphology of Trichophyton mentagrophyte

b) LPCB mount showing microscopic morphology of Trichophyton rubrum

c) LPCB mount showing microscopic morphology of Trichophyton verrucosum

d) Microscopic morphology of Microsporum gypseum

Table.4 Dermatophytes (Microsporum species) isolated from different clinical types: n (%)

Clinical types ↓	**Species→** ***M.audounii***	***M.gypseum***	***M. canis***	***M. praecox***	***M. nanum***	***M. ferruginium***	**Total**
T.unguium	0	0	0	0	0	0	0
T.capitis	6	10	2	1	0	1	20
T.corporis	2	0	1	1	1	0	5
T.pedis	0	0	0	0	0	0	0
T.cruris	0	0	0	0	0	0	0
T.manum	0	0	0	0	1	0	1
T.faciei	0	0	0	0	0	0	0
Total	8(4·23)	10(5·29)	3(1·58)	2	2	1	26(13·7)

Table.5 Dermatophytes (Epidermophyton species) isolated from different clinical types: n (%)

Clinical types ↓	**Species→** ***E. floccosum***	***E. stockdale***	**Total**
T.unguium	4	1	5
T.capitis	0	0	0
T.corporis	2	1	3
T.pedis	0	0	0
T.cruris	0	0	0
T.manum	0	0	0
T.faciei	0	0	0
Total	6 (3.17)	2(1.05)	8(4.23)

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